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LABORATORY-ACQUIRED ROCKY MOUNTAIN SPOTTED FEVER: THE HAZARD OF--ETC(U)
APR 77 C N OSTER, D S BURKE, R H KENYON

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Laboratory-Acquired Rocky Mountain Spotted Fever:
The Hazard of Aerosol Transmission

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RUNNING HEAD: AEROSOL HAZARD OF RMSF

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

28 April 1977

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LABORATORY-ACQUIRED ROCKY MOUNTAIN SPOTTED FEVER:

THE HAZARD OF AEROSOL TRANSMISSION

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RUNNING HEAD: AEROSOL HAZARD OF RMSF

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Abstract Nine patients with laboratory-acquired Rocky Mountain spotted fever (RMSF) were seen during the period 1971 to 1976. Investigation of each case revealed either definite or probable exposure to an aerosol containing infectious rickettsiae; in no case was there evidence of parenteral exposure either by accidental self-inoculation or by tick bite. These illnesses are believed to represent RMSF acquired via the respiratory route; this report emphasizes the aerosol hazard of Rickettsia rickettsii in the laboratory and discusses the possibility of respiratory transmission of RMSF in nature. Cell-mediated immunity, as measured by lymphocyte transformation to rickettsial antigen, was detected in these patients and was correlated with serologic studies.

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Rocky Mountain spotted fever (RMSF), an infectious disease caused by Rickettsia rickettsii, is endemic throughout the continental United States. Transmission of the disease to humans occurs via the bite of an infected arthropod, the dog tick, Dermacentor variabilis, in the eastern United States, or the wood tick, Dermacentor andersoni, in the western United States. Transmission can also occur by accidental self-inoculation of infectious material in both laboratory^{1,2} and clinical³ settings. Although aerosol inoculation of monkeys provides a well-studied animal model for RMSF,^{4,5} there are only three previously reported instances of laboratory acquired disease for which a careful investigation failed to disclose evidence of parenteral transmission and were believed to have been acquired by inhalation of infectious aerosols.^{1,2} From 1971 to 1976 members of the professional and support staff of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) were engaged in research into the pathogenesis and immunology of rickettsial diseases, with development of an effective vaccine against RMSF as a major objective. During this period there were nine cases of laboratory-acquired RMSF which we believe were acquired via aerosols. Additionally, personnel studying R. rickettsii in the laboratory demonstrate immunologic indicators of exposure to this organism even in the absence of clinical illness. These observations are evidence for the hazard of aerosol transmission of R. rickettsii in the laboratory.

Relatively little is known of cell-mediated immunity in rickettsial diseases. Wisseman et al.⁶ have demonstrated delayed cutaneous hypersensitivity to formalin-killed suspensions of Rickettsia mooseri and Rickettsia prowazeki in humans who either had

epidemic or endemic typhus, or were vaccinated with the attenuated living E strain of R. prowazeki. Coonrod and Shepard⁷ reported in vitro lymphocyte transformation (LT) to rickettsial antigens in humans after infection or vaccination with either typhus or spotted fever group organisms and noted some correlation of LT with complement-fixing antibody titer. The occurrence of these nine cases of laboratory-acquired RMSF afforded us the opportunity to monitor cell-mediated immunity, as measured by in vitro LT to R. rickettsii antigen, and correlate this response with serologic tests of rickettsial antibodies.

MATERIALS AND METHODS

Patients

The records of all patients with a diagnosis of RMSF from 1971 to 1976 were reviewed. All patients with a clinical picture compatible with RMSF, and either four-fold rises of specific antirickettsial antibody titers as measured by microagglutination (MA) or immunofluorescent antibody techniques (IFA), or isolation of R. rickettsii from a pretreatment blood sample are included.

Studies of Personnel

All personnel who worked in the laboratories where R. rickettsii was employed were bled in January, 1977 as part of routine ongoing occupational health screening. MA, IFA and LT tests to R. rickettsii antigen were performed. None of these personnel had a history of clinical RMSF. Personnel working in other laboratories at USAMRIID served as controls.

Complement-Fixation (CF) Test

Tests were kindly performed by Dr. Charles Shepard, Leprosy and Rickettsia Branch, Virology Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia.

Microagglutination (MA) Test

Tests for RMSF antibody were performed as described by Fiset et al.⁸ Antigen was prepared from the Sheila Smith strain of R. rickettsii grown in duck embryo cell (DEC) cultures. After differential centrifugation rickettsiae were inactivated with 0.1 per cent formalin and extracted twice with ethyl ether to remove egg lipids. The antigen suspensions were standardized to contain 1 mg rickettsiae per milliliter. Antigen-antiserum suspensions were incubated at 20°C for 18 hours after which time 25 µl of 0.02 per cent acridine orange was added. Results were recorded at 24 hours.

Weil-Felix (WF) Test

Proteus OX2 and OX19 slide antigen, tube antigen, and positive antiserum were purchased from Difco Laboratories, Detroit, Michigan. Serum titrations were performed in microtiter plates with 200 μ l of 1:8 saline dilution of slide antigen in each well. Sealed plates were incubated in a 37°C water bath, and agglutination was recorded after 4 hours. The tube test was performed as described by Weil and Felix.⁹

Indirect Fluorescent Antibody (IFA) Test

IFA tests were performed using the procedure described by Kenyon et al.¹⁰

Rickettsial Isolation

Blood obtained from patients on admission, before antibiotic treatment was initiated, was injected intraperitoneally into male, Hartley strain guinea pigs for rickettsial isolation as described by Elisberg and Bozeman.¹¹

Lymphocyte Transformation

Blood for leukocyte cultures was obtained from patients and laboratory personnel. Leukocyte suspensions separated from heparinized blood were used in tests for specific in vitro lymphocyte transformation essentially as described by Marker and Ascher.¹² The antigen employed was formalin-killed, DEC culture-grown R. rickettsii purified by rate zonal centrifugation and standardized by particle count¹³ at 5×10^7 rickettsiae per milliliter.

Statistics

The t-test for independent samples was used when comparing MA, IFA, and LT data of the vaccinated personnel and controls, and the MA data of unvaccinated personnel and controls. For this calculation, all data

were log-transformed, and negatives were assigned a value one-half of the lowest positive value for that particular test. Wilcoxon's rank sum test was employed for comparison of IFA and LT of unvaccinated personnel and controls. MA and IFA are compared to LT of patients and personnel using linear regression analysis of log-transformed data.

CASE REPORTS

PATIENT 1. On September 5, 1971, a 22-year-old white male laboratory technician, on leave from this laboratory, was admitted to the hospital at Fort Dix, New Jersey, with a six-day history of headache, chills, fever, stiff neck, myalgia, arthralgia, and chest pain. One day before admission a rash had developed on his arms and legs.

On admission, the patient was acutely ill with a temperature (T) of 39.8°C (103.6°F). Examination disclosed bilateral cervical adenopathy, a spleen palpable 4 cm inferior to the left costal margin and an erythematous maculopapular rash over the extremities and trunk. Admission laboratory data included a hematocrit (HCT), 42 per cent, white blood cell count (WBC) 7,300, with 70 per cent polymorphonuclear leukocytes (PMN), 22 per cent lymphocytes. Urinalysis, chest x-ray, febrile agglutinins, liver function tests, and heterophile antibody were all unremarkable. Routine cultures of the throat, urine, and blood were negative.

The patient was started on tetracycline hydrochloride, 2 g per day orally, and improved rapidly. He was afebrile within 48 and asymptomatic within 72 hours. At the time of discharge on the fourth hospital day, physical examination was normal except for the fading rash. Data confirming the diagnosis of RMSF are included in Table 1.

The patient worked as a technician in the laboratory. His work included inoculating and harvesting R. rickettsii from embryonated eggs and tissue cultures. He could recall no laboratory accident or break in technique; his only known exposure was daily work with materials containing high titers of R. rickettsii.

PATIENT 2. On January 31, 1972, a 20-year-old white male laboratory technician was admitted to the medical ward of this Institute with possible RMSF. Seven days before admission he had developed fever. Examination at that time revealed only right posterior auricular adenopathy; no treatment was administered. Three days later he returned with malaise, fever, sore throat, and diffuse myalgia and arthralgia. Physical examination revealed a macular erythematous rash on his hands and arms. He was afebrile. Complete blood count (CBC) was normal and a "Mono spot" test was negative. A throat culture grew normal flora. Two days before admission, he saw a private physician, who prescribed ampicillin, promethazine, and analgesics. These medications provided no relief, and he returned to the dispensary and was admitted for evaluation.

On admission the patient was acutely ill with temperature of 39.4°C (103°F). Examination revealed a diffuse maculopapular erythematous eruption, including the palms and soles, but sparing the face. There was a grade III/IV systolic ejection murmur at the lower left sternal border. The liver was felt at the right costal margin and was mildly tender. The spleen tip was felt at the left costal margin. Laboratory tests, including CBC, electrolytes, blood urea nitrogen (BUN), liver function tests, chest x-ray, and cultures of the throat, blood, and urine were unremarkable.

Treatment with tetracycline, 2 g per day orally, was begun. The patient became afebrile on the third hospital day. Symptoms resolved by the fifth hospital day, and he was discharged.

Data confirming the diagnosis of RMSF are included in Table 1.

The patient had worked in the laboratory harvesting yolk sacs from embryonated eggs infected with R. rickettsii for only one day, 13 days before the onset of his illness. He indicated that there

had been no accident or break in laboratory technique. His only known exposure was working with rickettsiae-laden material.

PATIENT 3. A 34-year-old white male veterinarian was seen in the USAMRIID dispensary on September 20, 1974, complaining of fatigue, myalgia, severe frontal headache, and fever for one day. In 1969 he had received the primary series (three injections at weekly intervals) of a commercial (Lederle) RMSF vaccine.

On physical examination, the patient had a temperature of 37.8°C (100°F), pulse, 88 per minute, respirations, 24 per minute, and blood pressure of 122/74 mm Hg. The patient did not appear extremely ill; he had no abnormalities on examination. His HCT was 45 per cent, WBC 5,100 with a normal differential, erythrocyte sedimentation rate (ESR) 10, platelet count 300,000, prothrombin time (PT) 14 seconds (control 12 seconds), partial thromboplastin time (PTT) normal, fibrinogen 355 mg per deciliter, BUN 21, and creatinine 1.0. Liver function tests, chest x-ray and ECG were normal. Because of suspected RMSF, the patient was started on tetracycline, 4 g per day by mouth, and followed as an outpatient. Forty-eight hours later he developed a faint macular erythematous rash, first on the arms, including the palms, and then spreading to the trunk and legs. This rash faded over the following 48 hours. At this time the patient was afebrile and asymptomatic, except for residual weakness and fatigue.

Laboratory data confirming the diagnosis of RMSF are included in Table 1.

Nine days before onset of this illness the patient and two co-workers were injecting guinea pigs with a yolk-sac suspension of R. rickettsii containing about 10^8 organisms per milliliter. While injecting one animal intradermally, the needle "popped" off the syringe

and the contents were sprayed into the air and spattered onto the hands and faces of the three individuals. Safety glasses prevented conjunctival contamination. All immediately washed themselves and then cleaned up the spill. Two co-workers elected to take prophylactic tetracycline and did not become ill or develop antibody titers to RMSF. The patient declined prophylaxis as he believed the previous RMSF vaccination would offer protection against disease.

PATIENT 4. A 21-year-old white male laboratory technician was admitted to the medical ward of the Institute on September 28, 1974. Three days before admission, the patient developed anorexia, malaise, headache, and myalgia, especially severe in both calves. One day before admission he experienced several shaking chills and developed a fever.

On admission the patient was acutely ill with temperature of 38.2°C (100.8°F), blood pressure, 104/70 mm Hg, respirations, 20 per minute, and pulse 100 per minute. Examination revealed a flushed face, inflamed pharynx, left anterior cervical adenopathy, and bilateral calf tenderness. Laboratory data included HCT 45 per cent, WBC 5,400 with a normal differential, ESR 11, and a normal chest x-ray. Routine throat and blood cultures were negative.

The patient was started on tetracycline 2 g per day by mouth. By the fourth hospital day, the patient felt much improved, and his temperature had returned to normal. This patient had no rash during his illness.

Laboratory data confirming the diagnosis are included in Table 1.

The patient recalled an accident resulting in heavy aerosol exposure on September 20, 1974. While injecting a yolk-sac suspension

of R. rickettsii into a vial, the stopper "popped" off, and the suspension was sprayed throughout the laminar flow hood at which he was working. Disregarding established procedure, he immediately opened the hood and spent approximately five minutes cleaning up the spill. This finished, he closed the hood, but remained in the same room for another two hours. He did not report the incident until he became ill.

PATIENT 5. A 62-year-old white male animal handler was admitted to the medical ward of this Institute on February 20, 1975. He had had a primary series of a trivalent rickettsial vaccine (RMSF, Q fever, and epidemic typhus) in 1958. Two days before admission, the patient experienced abrupt onset of severe frontal headaches, dizziness, nasal congestion, and coryza. His temperature was 40°C (104°F). The next day he continued to experience severe headaches, giddiness, mild unproductive cough, nausea, myalgia, and shaking chills.

Physical examination revealed an acutely ill male, experiencing a shaking chill. His temperature was 40.3°C (104.6°F), pulse, 116 per minute, blood pressure, 130/90 mm Hg, and respirations, 20 per minute. His pharynx was mildly injected. There was a III/IV blowing systolic murmur at the cardiac apex which intensified with inspiration. The skin of the trunk showed livedo reticularis, but there was no rash. Admission laboratory values were: HCT 47 per cent, WBC 3,400 with 4 per cent metamyelocytes, 40 per cent bands, and 45 per cent PMN, ESR 32, platelet count 86,000, PT 16 sec (control 14 sec), PTT normal, and fibrinogen 457 mg per deciliter. Fibrin split products were not detected. Electrolytes, BUN, creatine, liver function tests, chest x-ray, ECG, throat and blood cultures, and serum complement levels were normal. The cerebrospinal fluid was normal.

The patient was treated with tetracycline, 4 g per day by mouth, aspirin, and intravenous fluids. He remained severely ill for 48 hours with a hectic fever and frequent shaking chills. On the second hospital day his HCT was 37 per cent, WBC 2,600, platelet count 55,000. A tourniquet test was positive, and fibrin split products were detected. His PT and PTT were normal and the fibrinogen 350 mg per deciliter. He had no clinical evidence of bleeding. On the third hospital day he developed a fine macular erythematous rash on the distal lower extremities. The next day the rash was petechial and involved the soles of his feet. On the third hospital day, because of persistent nausea and vomiting, intravenous tetracycline was begun. Within 24 hours he became afebrile, and recovery progressed uneventfully.

Studies documenting RMSF are seen in Table 1. Throat washings taken on the day of admission yielded influenza type A virus. CF titers to type A influenza increased from negative to 1:16 and to type A₂ from 1:4 to 1:32 over the course of his illness. He, therefore, had simultaneous infection with influenza and RMSF.

Careful investigation revealed no accident or break in technique. About one week before he became ill the patient recalled emptying a trash barrel containing a broken roller bottle which had been used for tissue culture of R. rickettsii. This may have exposed the patient to an infectious aerosol.

PATIENT 6. A 26-year-old white male laboratory technician was admitted to the USAMRIID medical ward on June 29, 1975. Three days before admission, he had developed a retro-orbital headache unresponsive to aspirin. Over the next two days he also developed myalgia, mild unproductive cough, nausea, anorexia, sore throat, chills, and vomiting.

On admission his temperature was 39.7°C (103.4°F), pulse 94 per minute, respirations, 12 per minute and blood pressure, 128/62 mm Hg. He appeared acutely ill. His skin was pale. There was mild pharyngeal inflammation, bilateral conjunctivitis, and hepatomegaly with mild right upper quadrant abdominal tenderness.

Admission laboratory data included: HCT 44 per cent, WBC 5,200 with 9 per cent bands, 71 per cent PMN, 12 per cent lymphocytes, 2 per cent monocytes, and platelets of 321,000. Electrolytes, BUN, creatinine, glucose, LFT, creatine phosphokinase, amylase, ECG, chest x-ray and urinalysis were unremarkable.

Data confirming the diagnosis of RMSF are included in Table 1.

Tetracycline, 2 g per day by mouth, was begun immediately. Recovery was uneventful with the exception of transient thrombocytopenia (nadir 150,000 on the fourth hospital day). At no time did he have a rash. He was discharged on the fourth hospital day.

The patient's work involved inoculating and harvesting R. rickettsii from embryonated eggs and tissue cultures. He had not noted any accident or break in technique.

PATIENT 7. A 23-year-old white male laboratory technician was admitted to the medical ward May 25, 1976, with a two-day history of fever to 40°C (104°F), chills, sweats, myalgia, and moderately severe frontal headaches. The day of admission he also complained of nausea, vomiting, "burning eyes," and photophobia. Admission physical examination revealed temperature 39.4°C (103°F), pulse, 120 per minute, blood pressure, 115/80 mm Hg and respirations, 15 per minute. The skin was warm and moist; there were several 2-5-mm erythematous macules on the anterior chest. The remainder of his

examination was unremarkable. Admission laboratory data included: HCT 47 per cent, WBC 7,800 with 12 per cent bands, 77 per cent PMN, 6 per cent lymphocytes, and 5 per cent monocytes, platelets 140,000, and routine urinalysis showed mild proteinuria and 15-18 WBC. The remainder of the admission laboratory data, including electrolytes, glucose, BUN, creatine, liver function tests, ESR, PT, PTT, clotting time, VDRL, chest x-ray, ECG, "Mono spot" test, were normal. Lumbar puncture, performed on the second hospital day because of persistent headache and stiff neck, was entirely normal. Routine cultures of the throat, urine, blood, and CSF were negative.

He was immediately begun on tetracycline, 500 mg every six hours, by mouth. On the second hospital day, a few more macules were noted on his chest; thereafter his rash faded quickly over 48 hours. His recovery was uneventful; he became afebrile by the fourth hospital day and was discharged on the fifth.

Data confirming RMSF are given in Table 1.

This technician worked primarily with Rickettsia tsutsugamushi, but uneventfully harvested R. rickettsii from cell culture six days before the onset of his illness. This had been his only recent exposure to R. rickettsii.

PATIENT 8. A 19-year-old white female laboratory technician was admitted to the USAMRIID medical ward May 29, 1976, with a 24-hour history of fever to 38.9°C (102°F), fatigue, myalgia, chills, sweats, and moderately severe frontal headache. Physical examination revealed temperature, 38.7°C (101.6°F), pulse, 120 per minute, blood pressure, 130/70 mm Hg, and respirations, 18 per minute. The patient was flushed and diaphoretic. There was a faint erythematous macular rash on her trunk and proximal extremities. Her admission laboratory data

included: HCT 40 per cent, WBC 7,900 with 6 per cent bands, 60 per cent PMN, 1 per cent eosinophils, 1 per cent basophils, 1 per cent monocytes, and platelets, 135,000. Urinalysis showed mild proteinuria and heavy ketonuria. Electrolytes, BUN, creatinine, glucose, liver function tests, chest x-ray, ECG, PT, PTT, and clotting time were all normal. Routine cultures of the throat, urine, and blood were negative.

The patient was treated immediately with tetracycline, 500 mg every six hours, by mouth. By the second hospital day the macular rash had spread to involve the whole body, including the palms and soles, sparing only the face. The rash faded quickly, disappearing by the fourth hospital day. At this time she was afebrile and symptom-free. She was discharged on the fifth hospital day.

Data confirming RMSF are shown in Table 1.

This patient worked daily with R. rickettsii. Her duties included growing, harvesting, and concentrating rickettsiae. She could recall no laboratory accident or break in technique. Her only documented exposure was simply working with materials containing high titers of R. rickettsii.

PATIENT 9. A 21-year-old male veterinary technician was admitted to the medical ward of this Institute on October 7, 1976. Seven days prior to admission, he was seen in the clinic complaining of myalgia and an unproductive cough. He was afebrile; no abnormalities were detected on examination. Two days later, while on leave, he developed chills, and occipital headache. October 3 he was admitted to a community hospital in Ohio. On admission there, his temperature was 38.2°C (100.8°F); it soon rose to 40.0°C (104°F). He had a rash on his trunk and extremities which was described as "red and macular." Laboratory findings included:

HCT 40 per cent, WBC 3,000 with 10 per cent bands, 64 per cent PMN, 20 per cent lymphocytes, 2 per cent monocytes and 4 per cent eosinophils, platelets of 52,000, sodium 131 mEq per liter, chloride 103 mEq per liter potassium 3.4 mEq per liter and bicarbonate 23 mEq per liter. Cold agglutinins were positive at a 1:8 dilution. Blood cultures, throat culture and a "Mono spot" test were negative.

The patient was initially treated with procaine penicillin, but was begun on tetracycline 1 g per day, intramuscularly, on October 6. He remained febrile and was transferred to this Institute on October 7.

On admission his temperature was 38.3°C (101°F). Examination revealed a petechial rash involving the trunk and extremities, including the soles of the feet, but sparing the palms and face. Shotty cervical and axillary lymphadenopathy and a spleen tip palpable at the left costal margin were noted. Occasional rales were heard at the right base. Laboratory data included: HCT 40 per cent WBC 6,000 with 3 per cent bands, 53 per cent PMN, 43 per cent lymphocytes, 1 per cent eosinophils and 2 per cent monocytes. The platelet count was 152,000. Electrolytes, BUN, creatinine, urinalysis, PTT, PT, liver function tests, and ECG were normal. Small bilateral pleural effusions were seen on chest x-ray and lateral decubitus views. The patient was immediately begun on intravenous tetracycline, 500 mg every 6 hours, and recovered uneventfully. The pleural effusions cleared over 1 to 2 weeks. Laboratory data confirming the diagnosis are presented in table 1.

The patient was not involved in any studies with rickettsiae; however, one week prior to onset of his illness he spent approximately 15 minutes in a laboratory chatting with another technician who was pipetting R. rickettsii in a laminar flow hood. This was his only known exposure.

RESULTS

Diagnostic Studies

Table 1 depicts the results of Weil-Felix (WF), complement fixation (CF), immunofluorescent antibody (IFA), lymphocyte transformation (LT), and rickettsial isolation studies as performed on our patients. The WF OX19 gave diagnostic (four-fold or greater) titer rises in five of six patients, the OX2 in one of five, the CF in five of nine, the MA in five of eight, and the IFA in all of eight. LT data are expressed as a stimulation index, that is the ratio of counts per minute of antigen stimulated cultures to counts per minute of cultures with no antigen. The convalescent LT was positive in six of seven patients tested (the 95 per cent confidence limits of unvaccinated individuals in 0.2-9.9, and of vaccinated [Lederle] individuals, 0.2-22.9). Rickettsial isolation was positive in all patients tested.

Survey of Personnel

Table 2 depicts the results of MA, IFA, and LT of personnel working in the Rickettsiology Division laboratories. Four of 13 had specific rickettsial antibody as measured by MA (normal ≤ 8), and nine of 13 as measured by IFA (normal negative at 1:10 dilution). Only two of 13 had positive LT. Overall, nine of 13 personnel demonstrated one or more indices of immunity to R. rickettsii. As a group, vaccinated personnel of the Rickettsiology Division had significantly higher IFA titers and LT than vaccinated controls who had neither clinical RMSF nor worked with R. rickettsii in the laboratory. Unvaccinated personnel did not differ significantly from controls.

Using paired data from all personnel, exposed and unexposed, vaccinated and unvaccinated, LT was compared with MA and IFA using

log/log linear regression analysis. LT showed significant correlation with MA (correlation coefficient 0.17, slope 0.18, Y-intercept 0.39) at a P value of 0.05. There was no significant correlation of LT with IFA.

DISCUSSION

In 1971 only one investigator and one technician were involved in rickettsial studies in this laboratory. By 1974, this number had increased to seven investigators and ten technicians involved in studies of R. rickettsii primarily concerning research, development, and evaluation of a new type of RMSF vaccine prepared from cell culture grown organisms. Paralleling this increase in personnel working with rickettsiae was a concomitant increase in the incidence of laboratory-acquired RMSF. The nine patients with laboratory-acquired illness reported here are of interest because the circumstances surrounding their occurrence strongly suggest that disease was the result of inhalation of an infectious aerosol, a mode of transmission rarely recognized for RMSF.

As an obligate, intracellular parasite; R. rickettsii is sensitive to the effects of drying, ultraviolet irradiation, and heat. Natural transmission to man is via the bite of a tick vector. It can also be transmitted by accidental inoculation with a contaminated needle.^{1,3} During the period covered in this report, there was no RMSF secondary to accidental self-inoculation despite the occurrence of nine such accidents since January, 1975 (no records were kept prior to this date). This is believed to be due to the fact that all personnel immediately report such obvious exposure, and are treated prophylactically with tetracycline, 250 mg four times per day for five days. No person treated prophylactically, developed a significant serologic titer rise (subclinical RMSF), or overt illness.

That experimental RMSF can occur after aerosol challenge is well documented.^{4,5} The disease induced by aerosol exposure of monkeys is

similar clinically and pathologically to that seen when monkeys are challenged by other routes and resembles naturally-acquired disease in man. Disease in our patients also closely resembled naturally acquired RMSF, although two points deserve comment. Only five of our cases had prominent rash, while two had an evanescent macular rash, and two had no rash at all. Hattwick et al.¹⁴ reported only 55 of 1522 (3.6 per cent) cases of RMSF had no rash. Perhaps our high index of suspicion resulted in early treatment and consequent ablation of rash; conversely, our interest may have resulted in diagnosis of non-exanthematous cases which otherwise would not have been recognized. Secondly, six of our patients had symptoms or signs of upper respiratory tract involvement (one patient had coincidental influenza), suggesting local invasion of the respiratory tract by the aerosolized rickettsiae. Only one patient had physical and radiographic evidence of lower respiratory tract involvement.

Our experience suggests that laboratory personnel working with rickettsiae are frequently exposed despite careful adherence to established procedures for handling hazardous organisms.¹⁵⁻¹⁷ The results of such exposure are not uniform among our personnel, but rather seem to follow two patterns. First, older personnel, who began working in these laboratories before 1971, were vaccinated with the commercial (Lederle) RMSF vaccine. They now have high levels of immunity, significantly exceeding the vaccinated controls, and none ever had clinical RMSF. Dupont et al.¹⁸ demonstrated the lack of protective efficacy of the commercial vaccine in the specific instance when it is given as three weekly injections, and followed by intradermal challenge with virulent rickettsiae three to 17 months later. However, in our group of vaccinated personnel, most of whom received the three

primary injections and several subsequent boosters, the vaccine seems to have induced protective immunity to the low level aerosol challenges generated by laboratory procedures. Rather than causing illness in this group, these repetitive challenges have apparently served to boost vaccine-induced immunity. Newer personnel, those arriving since 1971, when vaccination of at-risk personnel was discontinued, have established a different response pattern. They arrive with no existing immunity to RMSF, and when exposed to the aerosols generated during their routine laboratory procedures, develop clinical RMSF. Only two vaccinated patients deviated from these patterns, and developed clinical illness. However, both had received only the primary immunizations with no booster immunizations. Also, one of them (patient 3) undoubtedly was exposed to a very large challenge dose of rickettsiae which may have overwhelmed whatever protective immunity he had.

We have observed no secondary spread of RMSF, reinforcing the concept that this disease is not directly transmissible from person to person, but there are several facts which suggest that person-to-person spread may be possible. First, RMSF can be transmitted via aerosol, and the infectious dose necessary to cause infection by this route may be very small.⁴ Second, pathological respiratory tract involvement has been demonstrated in both animals¹⁹ and man.²⁰ Hattwick et al.²¹ have reported clinical pneumonitis in 12 per cent of 338 cases. Johnson and Kadull¹ reported one patient from whom they were able to isolate rickettsiae in pharyngeal washings. Third, six of our patients had symptoms or signs of upper respiratory tract involvement, including three with a cough. Thus, there is a potential for direct person-to-person spread via infectious aerosol produced

by the cough of a patient with respiratory tract involvement. Indeed, we cannot exclude this possible mode of transmission between patients 7 and 8, although it is more probable that each was directly exposed in the laboratory. Of all reported cases of RMSF 4.4 per cent occurred in individuals living in the same residence,¹⁴ and there are many reports of concurrent cases within families involving as many as eight individuals.^{22,23} Direct person-to-person respiratory spread of RMSF is an intriguing, although unproven, explanation for these concurrent cases.

Having recognized the aerosol hazard of laboratory work with R. rickettsii, we would give prophylactic tetracycline treatment to all personnel who have had an overt aerosol exposure. Additionally, because RMSF can occur without recognized exposure, we recommend that any suspicious febrile illness in personnel working with rickettsiae also be treated. Ultimate control of laboratory-acquired RMSF, however, will await the development of a more effective vaccine. A new vaccine,²⁴ developed in this laboratory, has shown efficacy in animal studies,²⁵ and has undergone initial safety and immunogenicity studies in man.²⁶

Coonrod and Shepard⁷ have reported lymphocyte blastogenic responsiveness in humans to rickettsial antigens after both vaccination and disease. We were not able to demonstrate increased responsiveness to R. rickettsii antigen in individuals previously vaccinated but not working with the organism. However, we were able to demonstrate significantly higher levels of responsiveness in vaccinated personnel currently working with R. rickettsii, and after clinical RMSF in six of seven patients. Four of these patients were studied only after their illness, and we are unaware of the status of their responsiveness before illness. In our last three patients,

lymphocyte responses were measured serially. Patient 7 had not developed responsiveness when last measured one month after his illness, while Patients 8 and 9 developed responsiveness more than a month after their illnesses. We are unable to offer a satisfactory explanation for the lack of responsiveness of Patient 7. Perhaps he may have developed a positive response later, but his departure from this laboratory prevented further testing. Certainly LT compares favorably to other currently available diagnostic techniques for RMSF, and may be a sensitive indicator of cellular defenses required for protection against these intracellular parasites.

Diagnosis of RMSF in clinical practice most often depends upon CF or WF tests; rarely, rickettsial isolation is performed. Hattwick et al.¹⁴ recently reported that those cases confirmed only by WF were indistinguishable, clinically or epidemiologically, from CF and rickettsial isolation-confirmed cases, demonstrating the usefulness of the WF test. They suggest, however, that due to greater specificity and sensitivity, "the complement fixation test . . . where available, should be used in preference to the Weil-Felix test."¹⁴ Our data do not confirm this preference for the CF test. In our hands the CF test was negative in all patients; when repeated at the Center for Disease Control, only five of the nine patients had four-fold or greater rises in the CF titer. The CF test was less sensitive than either MA or IFA, and confirms similar observations made by Philip et al. of the Rocky Mountain Laboratories.²⁷ Consequently, we do not suggest reliance on the complement fixation test alone; the Weil-Felix test should be included in all cases of suspected RMSF. If possible, the MA, IFA, LT, or rickettsial isolation should also be performed.

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Table 1. Studies* Establishing the Diagnosis of Rocky Mountain Spotted Fever

Patient Number	Reciprocal titer														Rickettsial Isolation
	OX19		OX2		CF		MA		IFA		LT				
	A	C†	A	C	A	C	A	C	A	C	A	C			
1	Neg§	ND¶	Neg	ND	8	256	Neg	1024	<10	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	16	64	ND	ND	<10	160	ND	ND	ND	ND	+
3	4	32	ND	ND	16	32	Neg	Neg	<10	320	ND	111	ND	111	+
4	4	64	2	2	16	32	Neg	4096	<10	128	ND	48	ND	48	+
5	ND	ND	ND	ND	128	256	4	2048	<10	5120	ND	129	ND	129	+
6	Neg	Neg	Neg	Neg	8	16	Neg	Neg	<10	160	ND	89	ND	89	ND
7	Neg	32	8	16	<8	32	2	2	<10	80	2	6	2	6	+
8	Neg	8	0	16	16	128	2	32	<10	640	1	24	1	24	ND
9	8	128	4	8	8	32	4	256	<10	640	1	15	1	15	ND

*Weil-Felix, OX19, OX2; CF, complement fixation; MA, microagglutination; IFA, indirect fluorescent antibody; LT, lymphocyte transformation stimulation index.

[†]Acute

[‡]Convalescent

[§]Negative

[¶]Not Done

Table 2. Rickettsial Microagglutination (MA), Indirect Fluorescent Antibody (IFA), and Lymphocyte Transformation Stimulation Indices (LT) in Personnel Currently Working with Rickettsia rickettsii with no History of Clinical Rocky Mountain Spotted Fever

Subject	Months Since Last Vaccination	Total Number of Vaccinations	Reciprocal Titer		LT
			MA	IFA	
<u>Vaccinated Personnel</u>					
1	101	6	4	40	3.0
2	102	12	2	<10	5.9
3	79	3	8	320	7.0
4	100	7	512	10,240	10.6
5	72	5	16	1,280	65.7
6	86	7	8	1,280	13.0
7	100	3	4	80	28.0
8	100	7	32	80	3.7
Mean*	92.5	6.2	12	226	10.2
-1 SE	88.3	5.2	7	97	7.1
+1 SE	96.7	7.2	23	529	14.7
<u>Vaccinated Unexposed Controls</u>					
(Number of Controls)	(92)	(92)	(68)	(68)	(79)
Mean*	125.1	5.2	5	22	2.4
-1 SE	72.4	3.0	4	18	2.1
+1 SE	177.8	7.4	5	27	2.7
P value	NS	NS	NS	<0.005	<0.001
<u>Unvaccinated Personnel</u>					
9			4	<10	3.3
10			16	10	2.2
11			8	<10	1.1
12			2	<10	2.6
13			4	160	0.5
Mean*			5	12	1.6
-1 SE			4	6	1.1
+1 SE			8	22	2.2
<u>Unvaccinated Unexposed Controls</u>					
(Number of controls)			(63)	(61)	(86)
Mean*			4	6.0	1.6
-1 SE			3	5.5	1.4
+1 SE			4	6.5	1.7
P value			NS	NS	NS

*Geometric means are given for MA, IFA, and LT.